Centers for Disease Control and Prevention
Model Performance Evaluation Program

Mycobacterium tuberculosis
and
Nontuberculous Mycobacteria Drug Susceptibility Testing Program

Report of Results
for the Performance Evaluation Survey
Conducted During May 2011

UNITED STATES DEPARTMENT OF HEALTH AND HUMAN SERVICES

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MTB NTM DST Report for May 2011 Samples Survey

Purpose

The purpose of this report is to present the results of the Centers for Disease Control and Prevention (CDC) Model Performance Evaluation Program for *Mycobacterium tuberculosis* and Nontuberculous Mycobacteria Drug Susceptibility Testing (MPEP MTB NTM DST) survey sent to participants in May 2011.

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Introduction: Analyses of the May 2011 *M. tuberculosis* and Nontuberculous Mycobacteria Drug Susceptibility Test Results Reported by Participating Laboratories

This report analyzes the laboratory demographic information and drug susceptibility testing results reported to the Centers for Disease Control and Prevention (CDC) by participating laboratories for the panel of five *Mycobacterium tuberculosis* Complex[1] isolates shipped in May 2011. Panels were sent to 97 laboratories and 95 laboratories participated in evaluation of the panels.

This aggregate report is prepared in a format that will allow laboratories to compare their results with those obtained by other participants for the same strains using the same method, drug, and drug concentrations. We encourage circulation of this report to personnel who are involved with drug susceptibility testing, reporting, or interpreting for *M. tuberculosis* isolates.

CDC is neither recommending nor endorsing testing practices reported by participants. For approved standards, participants should refer to consensus documents published by the Clinical and Laboratory Standards Institute (CLSI), "Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard-Second Edition," M24-A2 (ISBN 1-56238-746-4).[1]

[1] Historically, the intent of the exercise was to assess performance using organism that were of Mycobacterium tuberculosis Complex and were non-tuberculous mycobacteria. Overtime, non-tuberculous mycobacteria have been dropped. Although it is possible that any of the eight species of Mycobacterium tuberculosis Complex could be present in the isolates selected, identification is not part of the panel selection nor the exercise and it is presumed M. tuberculosis is the dominant species represented. For these reasons and simplicity, we refer to M. tuberculosis throughout the report.

Susceptibility Testing Results for the *M. tuberculosis* Isolates Panel Shipped May 9, 2011

The table below provides the intended results of the panel shipment that was sent to participants in May 2011. Although CDC recommends broth-based methods for routine *M. tuberculosis* complex drug susceptibility testing, this table provides the results obtained by the reference agar proportion method, except in the case of pyrazinamide, where BACTEC™ MGIT™ 960 (MGIT™) was the testing method.

Isolate	Susceptibility Testing Results
A	Resistant to Ofloxacin (OFL) @ 2.0µg/ml
B	Resistant to Isoniazid (INH) @0.2µg/ml
C	Susceptible to all first-and second-line drugs
D	Resistant to Isoniazid (INH) @ 0.2µg/ml and 1µg/ml Resistant to Streptomycin (SM) @ 2.0µg/ml and 10.0µg/ml
E	Resistant to Rifampin (RMP) @ 1.0µg/ml

Descriptive Information About Participant Laboratories

Primary Classification

This report contains the drug susceptibility testing results submitted to CDC by 95 laboratories in 41 states and Puerto Rico.

The participants were asked to indicate the **primary classification** of their laboratory.
MPEP participants self-classified as

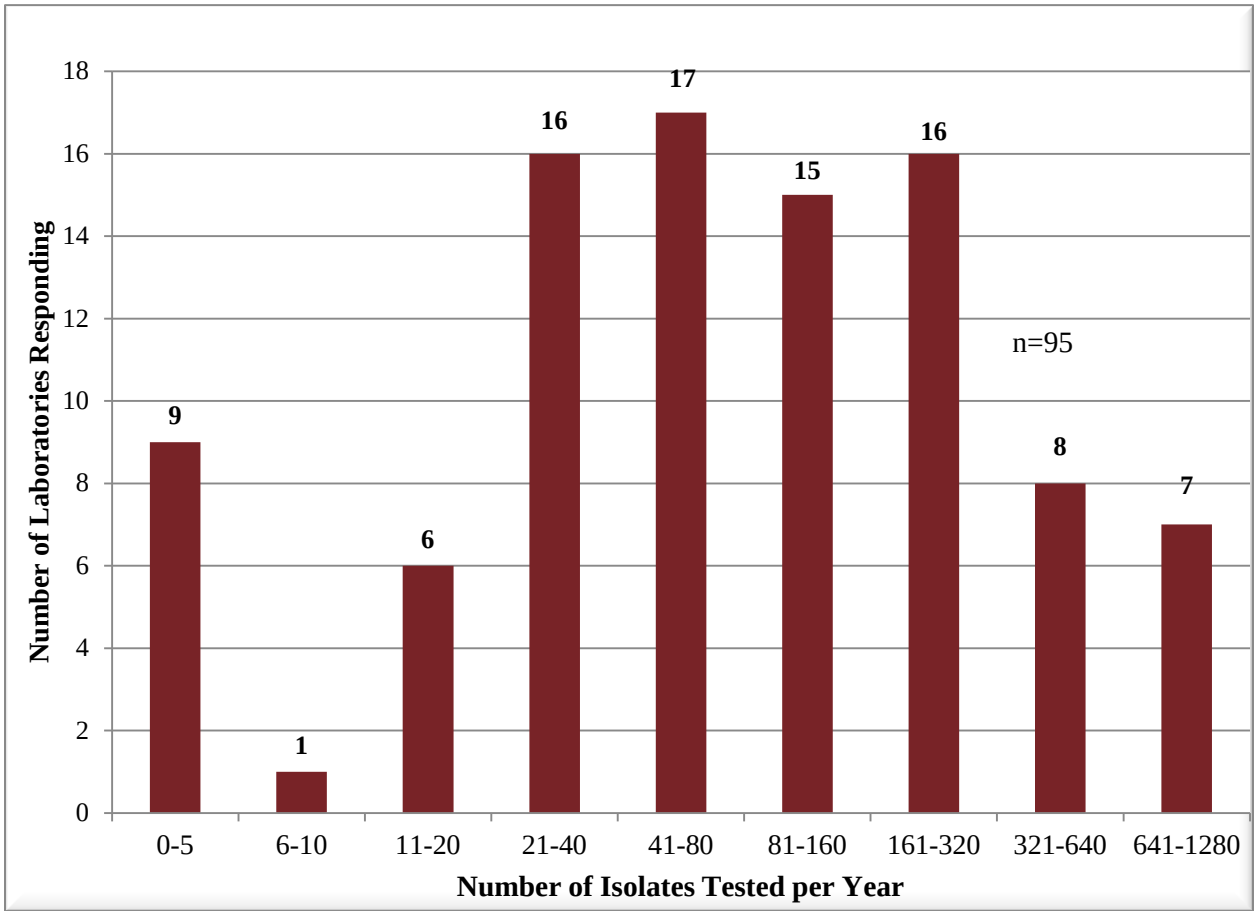
- 61 (64.2%): Health Department (city, county, state, regional, district, or national reference laboratory);
- 22 (23.2%): Hospital [city, county, district, community, state, regional, military, Veterans Administration, Federal government (other than military), privately-owned, university, HMO/PPO*-owned and operated, or religious-associated];
- 10 (10.5%): Independent [e.g., commercial, commercial manufacturer of reagents, HMO satellite clinic, reference laboratory (non-government affiliated)]; and
- 2 (2.1%): Other [Federal government research (nonmilitary)];

* HMO: health maintenance organization; PPO: preferred provider organization

Annual Number of *M. tuberculosis* Drug Susceptibility Tests Performed by Participants

Figure 1 shows the number of drug susceptibility tests performed on *M. tuberculosis* isolates by the 95 participants in one **calendar year,** January 1–December 31, 2010, excluding quality control isolates. The counts range from zero to 1,055. Sixteen (16) laboratories reported performing less than 21 drug susceptibility tests per year. To ensure testing proficiency, laboratories with low volumes are encouraged to consider referral of *M. tuberculosis* drug susceptibility testing.

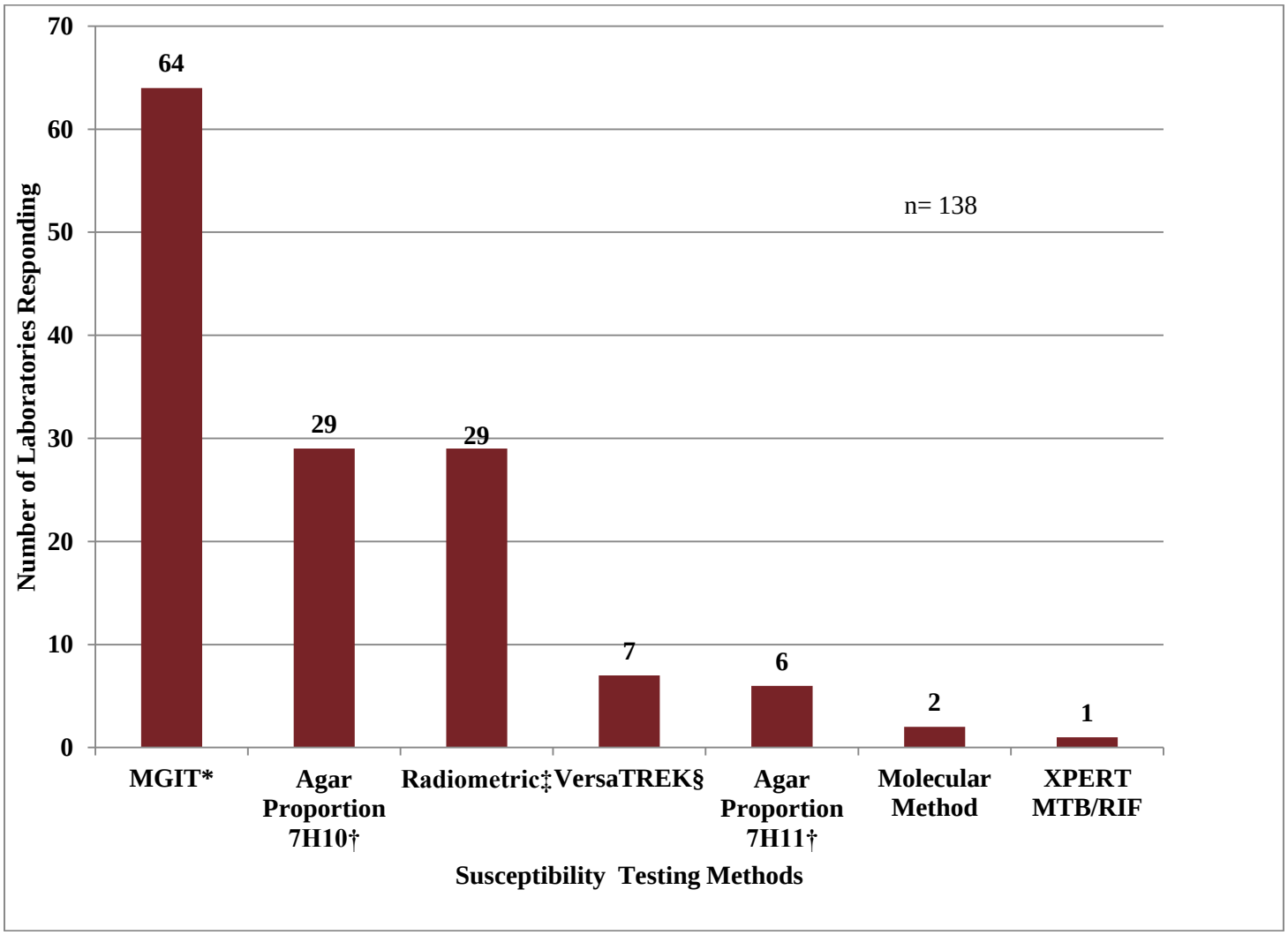
Figure 1: Distribution of the Annual Volume of *M. tuberculosis* Isolates Tested for Drug Susceptibility by Participants in the 2010 Calendar Year



Laboratory Susceptibility Testing Procedures Used by Participants

Participants were asked to report all *M. tuberculosis* susceptibility testing methods that were used to test these isolates. Fifty-eight laboratories used only one method for testing, whereas 31 laboratories used two methods, and six laboratories used three methods. Figure 2 shows the reported susceptibility methods.

Figure 2: Susceptibility Testing Methods Reported by Participant Laboratories

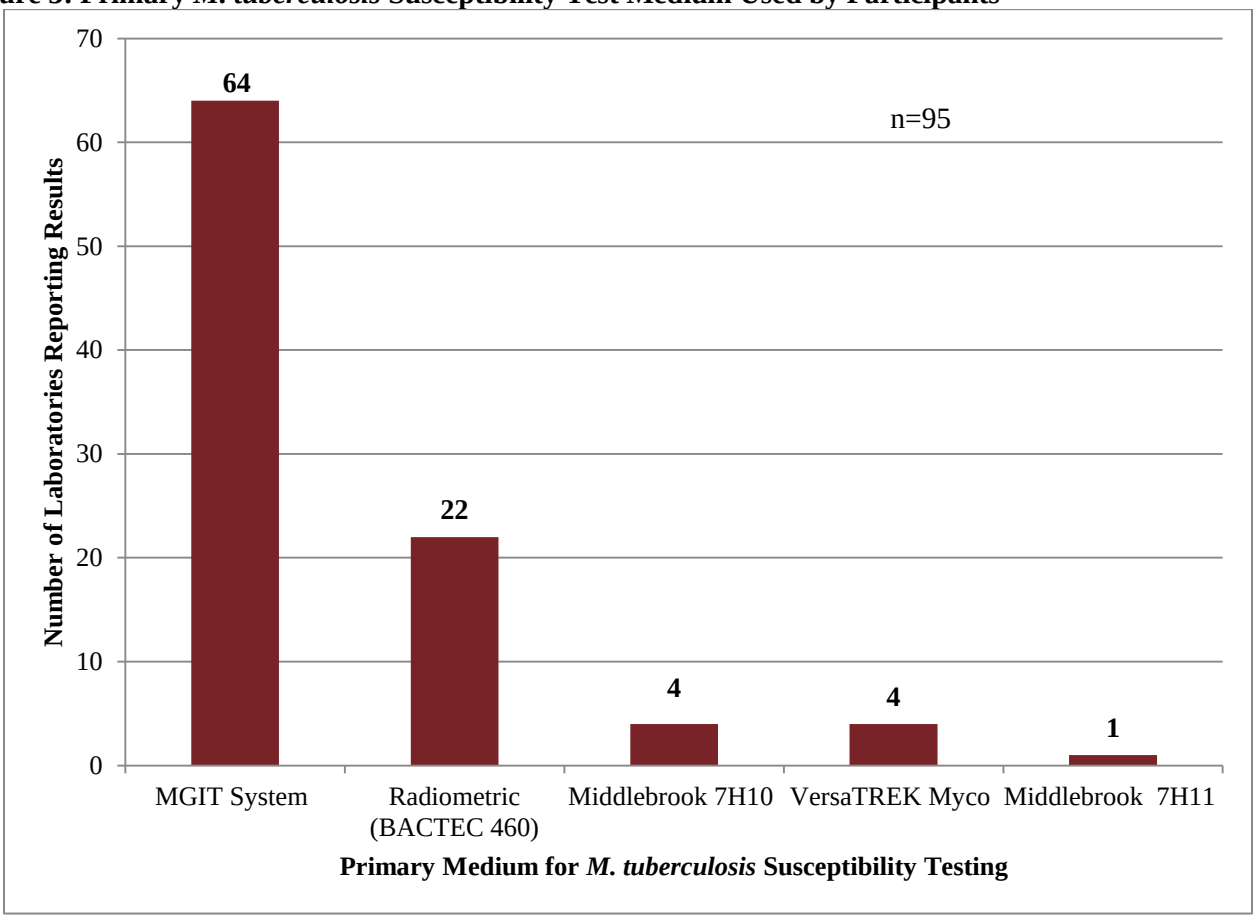


* MGIT™ Mycobacteria Growth Indicator Tube
† Agar proportion using Middlebrook medium
‡ Radiometric is BACTEC™ 460TB
§ VersaTREK® Myco Susceptibility Kit

The Primary *M. tuberculosis* Susceptibility Testing Media Used by Participants

Participants were asked to indicate the **primary** *M. tuberculosis* susceptibility test medium used by their laboratory for the isolates in the May 2011 shipment. Instructions were to select only one method as their primary method. Figure 3 shows the responses submitted by the 95 participants.

Figure 3: Primary *M. tuberculosis* Susceptibility Test Medium Used by Participants



Of the 64 laboratories that reported using MGIT™ as one of their methods for testing the MTB NTM DST isolates,

- 60 indicated that the MGIT™ method was their primary method for susceptibility testing;
- 2 laboratories indicated Agar proportion (AP) was their primary method using Middlebrook 7H10.

Of the 29 laboratories who reported using BACTEC™ 460TB as one of their methods for testing the isolates,

- 22 used this as their primary method;
- 6 used MGIT™ as their primary method; and
- 1 laboratory indicated AP was their primary method using Middlebrook 7H11.

Of the 34 laboratories who reported using AP as a method for testing the isolates,

- 5 used this as their primary method;
- 8 used BACTEC™ as their primary method;

- 20 used MGIT™ as their primary method;
- 1 used VersaTREK®.

All 4 laboratories who reported using VersaTREK® indicated that it is their primary method.

Nontuberculous Mycobacteria Drug Susceptibility Testing Capacity

When participating laboratories were asked if they performed susceptibility testing of Nontuberculous Mycobacteria (NTM),

- 21 (22%) responded "Yes" and
- 74 (78%) responded "No."

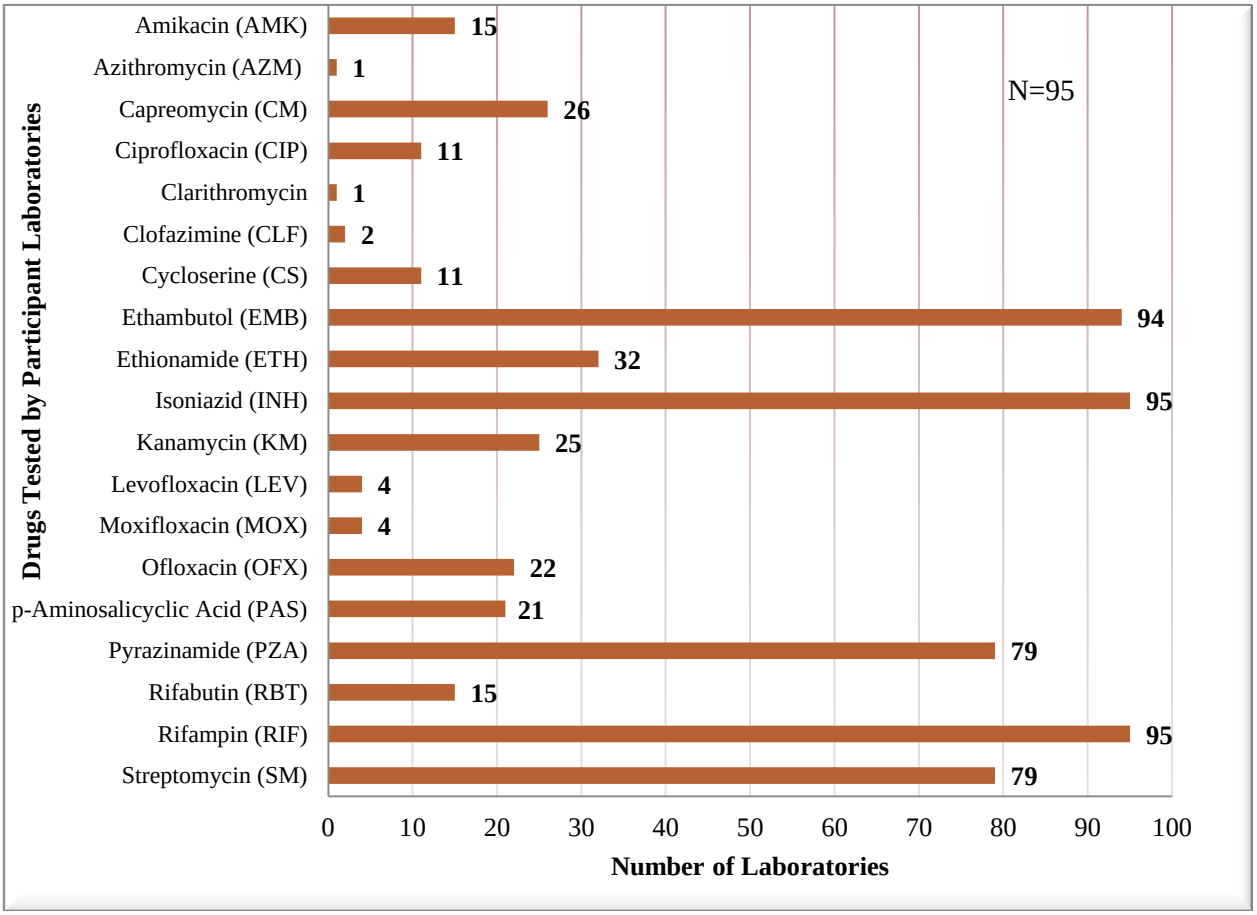
Forty-nine of the 77 laboratories (64%) that do not test NTM isolates in-house responded that they refer those isolates to another laboratory for drug susceptibility testing.

Antituberculous Drugs Used by Participants

CLSI recommends a full panel of first-line (primary) drugs (isoniazid [INH], rifampin [RMP], ethambutol [EMB], and pyrazinamide [PZA])1, because it represents a combination of tests that provides the clinician with comprehensive information related to the four-drug therapy currently recommended for treatment of most patients in the United States with tuberculosis. All participants tested three of the first-line drugs—INH, RMP, and EMB; 79 (83%) of these also tested PZA.

Figure 4 shows the number of laboratories testing each drug. The number at the right of each bar represents the number of laboratories that tested the drug.

Figure 4: Antituberculous Drugs Used by 95 Participants



Note: Providing test results for all drugs that are reported to CDC by participants should not be construed as a recommendation or endorsement for testing particular drugs or drug concentrations with *M. tuberculosis* isolated from patients. It is assumed that some of the drugs are being tested for research purposes or potential use in the few referral institutions that may treat patients with *M. tuberculosis* isolates resistant to almost all standard drugs. According to CLSI, "Second-line drugs may be tested simultaneously if mutations associated with INH and RMP resistance have been detected by molecular assays, or if epidemiological situations support the practice and resources are available. Second-line drugs, both traditional and newer agents, should be tested for isolates resistant to RMP or any two of the primary drugs. Isolates with monoresistance to the critical concentration of INH also should be tested for susceptibility to second-line agents if the clinician is planning to include a fluoroquinolone in the treatment regimen."1 Laboratories should not add drugs to their testing panel without consulting physicians with expertise in treating multidrug-resistant tuberculosis. Laboratories may contact their local tuberculosis control program for referrals to physicians expert in the treatment and care of tuberculosis.

Explanation of Tables 1 through 5

1. In the following tables, the shaded rows indicate critical concentrations for each test method. For each drug, the critical concentration is defined as the lowest concentration that inhibits 95% of "wild-type" strains of *M. tuberculosis* organisms that have not been exposed to the drug; but that simultaneously does not inhibit strains of the *M. tuberculosis* considered resistant that are isolated from patients who are not responding to therapy.[1]

2. The test results (S represents susceptible and R represents resistant) are listed in the appropriate columns along with a corresponding total number of tests (Sum column) to provide a denominator for determining the level of consensus. This report contains all results reported by participating laboratories, including many drug concentrations with only one result.

3. Participants should note that the CLSI approved standard "Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes," M24-A2 (ISBN 1-56238-746-4) CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA, 2011 recommends testing streptomycin as a second line drug and also adds ofloxacin and rifabutin to the list of recommended secondary drugs. For a complete list of drugs to be tested, consult the CLSI document M24-A2.[1]

4. Concentrations are listed in micrograms per milliliter (µg/ml).

5. A concentration of 0.00 is entered for results associated with genetic testing [Hain GenoType® MTBDR*plus* Assay or Hain GenoType® MTBDRsl Kit (HAIN Lifescience, Germany)] for which no drug concentration is required.

Isolate A, M. tuberculosis–susceptible to first-line drugs; resistant to Ofloxacin at 2.0µg/ml by Agar Proportion

All laboratories using AP, MGIT, and VersaTREK reported this isolate to be fully susceptible to the first-line drugs (INH, RMP, EMB, PZA) at all concentrations tested. However, of the 22 laboratories reporting MGIT results for RMP, one reported resistance to RMP at 2.0µg/ml

Ofloxacin

Fluoroquinolones (FQ) are important class of drugs to treat tuberculosis resistant to first-line drugs. They are the most commonly prescribed antibiotic class in the United States and they have the potential to become part of future first-line antituberculosis regimens.[2] Resistance to FQ is relatively low in strains of *M. tuberculosis* susceptible to first-line drugs but receipt of FQ before tuberculosis (TB) diagnosis is associated with a high risk of FQ-resistant TB and delays in diagnosis.[2,3]

Resistance to FQ has been mainly attributed to mutations in a 21-bp region of the *M. tuberculosis gyrA* gene, often called the quinolone resistance determining region (QRDR).[4]

All laboratories that reported agar proportion results for ofloxacin at the critical concentration of 2.0µg/ml or levofloxacin at the critical concentration of 1.0µg/ml reported resistance.

See Table 1 for the complete results submitted by all participants for Isolate A.

Table 1: Participant results for *M. tuberculosis*, Isolate A–resistant to Ofloxacin at 2.0µg/ml by AP

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Results*		
Drug	Conc.	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
Isoniazid	0.00										1		1
Isoniazid	0.10				23		23	61		61	4		4
Isoniazid	0.20	22		22				1		1			
Isoniazid	0.40				8		8	21		21	4		4
Isoniazid	1.00	23		23	1		1						
Isoniazid	4.00							1		1			
Isoniazid	5.00	4		4									
Rifampin	0.00										2		2
Rifampin	1.00	25		25	3		3	62		62	4		4
Rifampin	2.00				21	1	22	1		1			
Rifampin	5.00	3		3									
Pyrazinamide	20.00				1		1						
Pyrazinamide	100.00	1		1	16		16	59		59			
Pyrazinamide	300.00				1		1				3		3
Pyrazinamide	900.00				1		1						
Ethambutol	0.00										1		1
Ethambutol	2.50				21		21	1		1			
Ethambutol	5.00	21		21	3		3	59		59	5		5
Ethambutol	7.50	2		2	3		3	2		2			
Ethambutol	8.00										4		4
Ethambutol	10.00	9		9									
Streptomycin	1.00				1		1	46		46	1		1
Streptomycin	2.00	25		25	19		19	1		1	1		1
Streptomycin	4.00	1		1				6		6			
Streptomycin	6.00				2		2						
Streptomycin	10.00	21		21									
Ethionamide	1.25				1		1						
Ethionamide	2.50				1		1						
Ethionamide	5.00	19		19	3		3	2		2			
Ethionamide	10.00	4		4									
Kanamycin	0.00										1		1
Kanamycin	5.00	10		10	1		1						
Kanamycin	6.00	10		10									
Capreomycin	0.00										1		1
Capreomycin	3.00							2		2			
Capreomycin	5.00				4		4						
Capreomycin	10.00	18		18									
Cycloserine	30.00	8		8									
Cycloserine	60.00	1		1									

* VersaTREK®, Hain GenoType®, or Molecular Method

Table 1 continued: *M. tuberculosis*, Isolate A– resistant to Ofloxacin at 2.0µg/ml by AP

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Results*		
Drug	Conc.	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
p-Aminosalicylic acid	8.00	2		2									
p-Aminosalicylic acid	10.00	4		4									
Amikacin	0.00										1		1
Amikacin	1.50							2		2			
Amikacin	2.00	1		1									
Amikacin	2.50				1		1						
Amikacin	4.00	3		3									
Amikacin	5.00	1		1	1		1						
Amikacin	6.00	6		6									
Amikacin	12.00	2		2									
Ofloxacin	0.00										1		1
Ofloxacin	1.00		1	1		1	1						
Ofloxacin	2.00		15	15		3	3						
Ofloxacin	4.00	1		1		1	1						
Clofazimine	1.00	1		1									
Rifabutin	0.50	7		7	1		1						
Rifabutin	1.00	2		2	1		1						
Rifabutin	2.00	8		8									
Ciprofloxacin	1.00	1		1		1	1						
Ciprofloxacin	2.00		6	6		2	2						
Ciprofloxacin	4.00					1	1						
Levofloxacin	1.00		1	1									
Levofloxacin	1.50								2	2			
Levofloxacin	2.00				1	1	2						
Moxifloxacin	0.25								1	1			
Moxifloxacin	1.00	2	1	3				1		1			

* VersaTREK®, Hain GenoType®, or Molecular Method

Isolate B, *M. tuberculosis*–resistant to Isoniazid at 0.2µg/ml by Agar Proportion

Isoniazid

Isoniazid is the most widely used first-line anti-TB drug. It is the cornerstone of all effective regimens for the treatment of TB disease and latent infection. INH is a prodrug and is activated by the catalase-peroxidase enzyme KatG encoded by the *kat*G gene.[4,5] The target of activated INH is enoyl-acyl-carrier protein reductase (InhA) which is required for mycolic acid biosynthesis. There are two described mechanisms that account for the majority of INH resistance.[4] The most common method, mutations in *kat*G, is generally associated with high-level resistance to INH. Resistance to INH can also occur by mutations in the promoter region of the *inh*A gene which are generally associated with low-level resistance to INH and are less frequent than *kat*G mutations. DNA sequence analysis of *inh*A and *kat*G of Isolate B revealed a cytosine to thymine transition at the nucleotide positioned 15 bases upstream of the start codon (C(-15)T) in *inh*A; *kat*G was wild-type (i.e., no mutations were detected).

The recommended critical concentration and additional higher concentrations for testing INH using the AP method are, respectively, 0.2µg/ml and 1.0µg/ml. The equivalent concentrations for BACTEC™, MGIT™, and VersaTREK® are 0.1µg/ml and 0.4µg/ml. All participants tested this isolate with at least one concentration, but 2 laboratories (2 reporting MGIT™ results) did not report results at the critical concentration.

Ninety-four laboratories reported INH results for this isolate at the critical concentration. (Some laboratories submitted results for more than one method.) This isolate was reported resistant at the critical concentration of INH by method as follows:

- 92% (22/24) of the results when using AP;
- 100% (23/23) of the results when using BACTEC™;
- 100% (62/62) of the results when using MGIT™;
- 100% (4/4) of the results when using VersaTREK®.

The laboratory using Hain GenoType® MTBDR*plus* reported INH resistance.

Most laboratories reporting results at the higher concentrations of INH reported this isolate to be susceptible. This isolate was reported susceptible at the higher concentration of INH by method as follows:

- 100% (24/24) of the results when using AP;
- 100% (8/8) of the results when using BACTEC™;
- 88% (29/33) of the results when using MGIT™;
- 100% (4/4) of the results when using VersaTREK®.

Ethionamide

Ethionamide (ETH) is a second-line drug used to treat drug-resistant TB. It is a derivative of nicotinic acid and is bactericidal against *M. tuberculosis*. Like INH, ETH is a prodrug that is activated by a monooxygenase and it inhibits the same target as INH (*Inh*A).[4] Mutations in *inh*A confer resistance to ETH in addition to resistance to INH. ETH susceptibility testing is problematic.[6]

Twenty laboratories reported ETH results for this isolate at the critical concentration for AP (5.0µg/ml); 17 (85%) reported resistance.

See Table 2 for the complete results submitted by all participants for Isolate B.

Table 2: Participant results for *M. tuberculosis*, Isolate B–resistant to INH at 0.2µg /ml by AP

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Results*		
Drug	Conc.	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
Isoniazid	0.00											1	1
Isoniazid	0.10					23	23		62	62		4	4
Isoniazid	0.20	1	22	23		1	1		1	1			
Isoniazid	0.40				8		8	29	4	33	4		4
Isoniazid	1.00	24		24	2		2						
Isoniazid	4.00								1	1			
Isoniazid	5.00	3		3	1		1						
Rifampin	0.00										2		2
Rifampin	1.00	26		26	3		3	63		63	4		4
Rifampin	2.00				22		22	1		1			
Rifampin	5.00	3		3									
Pyrazinamide	20.00				1		1						
Pyrazinamide	100.00	1		1	16		16	59		59			
Pyrazinamide	300.00				1		1				3		3
Pyrazinamide	900.00				1		1						
Ethambutol	0.00										1		1
Ethambutol	2.50				21		21	1		1			
Ethambutol	5.00	22		22	3		3	60		60	5		5
Ethambutol	7.50	2		2	3		3	2		2			
Ethambutol	8.00										4		4
Ethambutol	10.00	11		11									
Streptomycin	1.00				1		1	47		47	1		1
Streptomycin	2.00	26		26	19		19	1		1	1		1
Streptomycin	4.00	1		1				6		6			
Streptomycin	6.00				2		2						
Streptomycin	10.00	21		21									
Ethionamide	1.25					1	1						
Ethionamide	2.50					1	1						
Ethionamide	5.00	3	17†	20	1	2	3		2	2			
Ethionamide	10.00	1	2	3					1	1			
Kanamycin	0.00										1		1
Kanamycin	5.00	9	1	10	1		1						
Kanamycin	6.00	11		11									
Capreomycin	0.00										1		1
Capreomycin	3.00							2		2			
Capreomycin	5.00				4		4						
Capreomycin	10.00	19		19									
Cycloserine	30.00	9		9									
Cycloserine	60.00	1		1									

* VersaTREK®, Hain GenoType®, or both

† Includes borderline results

Table 2 continued: *M. tuberculosis*, Isolate B–resistant to INH at 0.2µg /ml by AP

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Results*		
Drug	Conc.	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
p-Aminosalicylic acid	2.00	16		16									
p-Aminosalicylic acid	4.00				1		1						
p-Aminosalicylic acid	8.00	2		2									
p-Aminosalicylic acid	10.00	4		4									
Amikacin	0.00										1		1
Amikacin	1.50							2		2			
Amikacin	2.00	1		1									
Amikacin	2.50				1		1						
Amikacin	4.00	2		2									
Amikacin	5.00	1		1	1		1						
Amikacin	6.00	6		6									
Amikacin	12.00	2		2									
Ofloxacin	0.00										1		1
Ofloxacin	1.00	1		1	1		1						
Ofloxacin	2.00	14		14	3		3	1		1			
Ofloxacin	4.00	1		1	1		1						
Clofazimine	1.00	1		1									
Rifabutin	0.50	7		7	1		1						
Rifabutin	1.00	2		2	1		1						
Rifabutin	2.00	7		7									
Ciprofloxacin	1.00	1		1	1		1						
Ciprofloxacin	2.00	5		5	2		2						
Ciprofloxacin	4.00				1		1						
Levofloxacin	1.00	1		1									
Levofloxacin	1.50							2		2			
Levofloxacin	2.00				2		2						
Moxifloxacin	0.25							1		1			
Moxifloxacin	1.00	2		2									

* VersaTREK®, Hain GenoType®, or both

Isolate C, *M. tuberculosis*–susceptible to all first- and second-line drugs

This isolate is susceptible to all of the first- and second-line drugs.

Most participants (97.9%) reported this isolate susceptible to all drugs tested by all methods.

However, 2 different laboratories reported one instance of resistance:

- 1 laboratory using BACTEC 460 reported resistance to EMB at 2.5µg/ml; and,
- 1 laboratory using Agar Proportion (Middlebrook 7H10) reported resistance to RBT at 0.5µg/ml.

See Table 3 for the complete results submitted by all participants for Isolate C.

Table 3: Participant results for *M. tuberculosis*, Isolate C– Susceptible to all first-and second-line drugs

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Results*		
Drug	Conc.	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
Isoniazid	0.00										1		1
Isoniazid	0.10				23		23	62		62	4		4
Isoniazid	0.20	21		21				1		1			
Isoniazid	0.40				8		8	22		22	4		4
Isoniazid	1.00	22		22	1		1						
Isoniazid	4.00							1		1			
Isoniazid	5.00	3		3									
Rifampin	0.00										2		2
Rifampin	1.00	24		24	3		3	63		63	4		4
Rifampin	2.00				22		22	1		1			
Rifampin	5.00	3		3									
Pyrazinamide	20.00				1		1						
Pyrazinamide	100.00	1		1	16		16	59		59			
Pyrazinamide	300.00				1		1				3		3
Pyrazinamide	900.00				1		1						
Ethambutol	0.00										1		1
Ethambutol	2.50				20	1	21	1		1			
Ethambutol	5.00	20		20	3		3	60		60	5		5
Ethambutol	7.50	2		2	3		3	2		2			
Ethambutol	8.00										4		4
Ethambutol	10.00	9		9									
Streptomycin	1.00				1		1	47		47	1		1
Streptomycin	2.00	24		24	19		19	1		1	1		1
Streptomycin	4.00	1		1				6		6			
Streptomycin	6.00				2		2						
Streptomycin	10.00	20		20									
Ethionamide	1.25				1		1						
Ethionamide	2.50				1		1						
Ethionamide	5.00	18		18	3		3	2		2			
Ethionamide	10.00	3		3									
Kanamycin	0.00										1		1
Kanamycin	5.00	9		9	1		1						
Kanamycin	6.00	10		10									
Capreomycin	0.00										1		1
Capreomycin	3.00							2		2			
Capreomycin	5.00				4		4						
Capreomycin	10.00	17		17									
Cycloserine	30.00	8		8									
Cycloserine	60.00	1		1									

* VersaTREK®, Hain GenoType®, or both

Table 3 continued: *M. tuberculosis,* Isolate C–Susceptible to all first-and second-line drugs

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Results*		
Drug	Conc.	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
p-Aminosalicylic acid	2.00	14		14									
p-Aminosalicylic acid	4.00				1		1						
p-Aminosalicylic acid	8.00	2		2									
p-Aminosalicylic acid	10.00	4		4									
Amikacin	0.00										1		1
Amikacin	1.50							2		2			
Amikacin	2.00	1		1									
Amikacin	2.50				1		1						
Amikacin	4.00	2		2									
Amikacin	5.00	1		1	1		1						
Amikacin	6.00	6		6									
Amikacin	12.00	2		2									
Ofloxacin	0.00										1		1
Ofloxacin	1.00	1		1	1		1						
Ofloxacin	2.00	14		14	3		3						
Ofloxacin	4.00	1		1	1		1						
Clofazimine	1.00	1		1									
Rifabutin	0.50	6	1	7	1		1						
Rifabutin	1.00	2		2	1		1						
Rifabutin	2.00	7		7									
Ciprofloxacin	1.00	1		1	1		1						
Ciprofloxacin	2.00	5		5	2		2						
Ciprofloxacin	4.00				1		1						
Levofloxacin	1.00	1		1									
Levofloxacin	1.50							2		2			
Levofloxacin	2.00				2		2						
Moxifloxacin	0.25							1		1			
Moxifloxacin	1.00	2		2									

* VersaTREK®, Hain GenoType®, or both

Isolate D, *M. tuberculosis*–resistant to Isoniazid at 0.2µg/ml and 1.0µg/ml, Streptomycin at 2.0µg/ml and 10.0µg/ml by Agar Proportion

Isoniazid

94 laboratories reported INH results for this isolate at the critical concentration. (Some laboratories submitted results from more than one method.) This isolate was reported resistant to INH at the critical concentration by method as follows:

- 100% (24/24) of the results when using AP;
- 96% (22/23) of the results when using BACTEC™;
- 100% (62/62) of the results when using MGIT™;
- 100% (4/4) of the results when using VersaTREK®;

DNA sequence analysis of *inh*A and *kat*G of Isolate D revealed a G>C point mutation in the katG locus resulting in serine being replaced by threonine at codon 315 (Ser315Thr); *inhA* was wild-type (i.e., no mutations were detected).

The laboratory using Hain GenoType® MTBDR*plus* reported INH resistance.

All laboratories reporting results at the higher concentrations of INH reported this isolate to be resistant.

Streptomycin

Ninety-four laboratories reported SM results for this isolate at the critical concentration (2.0µg/ml for AP; 2.0µg/ml for BACTEC™, 1.0µg/ml for MGIT™). (Some laboratories submitted results from more than one method.) This isolate was reported resistant to SM by method as follows:

- 100% (28/28) of the results when using AP;
- 100% (19/19) of the results when using BACTEC™; and
- 100% (47/47) of the results when using MGIT™.

See Table 4 for the complete results submitted by all participants for Isolate D.

Table 4: Participant results for *M. tuberculosis*, Isolate D– resistant to Isoniazid at 0.2µg/ml and 1.0µg/ml, Streptomycin at 2.0µg/ml and 10.0µg/ml by AP

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Results*		
Drug	Conc.	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
Isoniazid	0.00											1	1
Isoniazid	0.10				1	22	23		62	62		4	4
Isoniazid	0.20		24	24		1	1		1	1			
Isoniazid	0.40					8	8		33	33		4	4
Isoniazid	1.00		25	25		2	2						
Isoniazid	2.00				1		1						
Isoniazid	4.00								1	1			
Isoniazid	5.00		4	4	1		1						
Rifampin	0.00										2		2
Rifampin	1.00	28		28	3		3	63		63	4		4
Rifampin	2.00				22		22	1		1			
Rifampin	5.00	4		4									
Pyrazinamide	20.00				1		1						
Pyrazinamide	100.00	1		1	16		16	58	1	59			
Pyrazinamide	300.00				1		1				3		3
Pyrazinamide	900.00				1		1						
Ethambutol	0.00										1		1
Ethambutol	2.50				21		21	1		1			
Ethambutol	5.00	22	2	24	3		3	60		60	5		5
Ethambutol	7.50	2		2	3		3	2		2			
Ethambutol	8.00										4		4
Ethambutol	10.00	12		12									
Streptomycin	1.00					1	1		47	47		1	1
Streptomycin	2.00		30	30		19	19		1	1		1	1
Streptomycin	4.00		2	2		1	1		10	10			
Streptomycin	6.00					3	3						
Streptomycin	10.00		25	25		1	1						
Ethionamide	1.25				2		2						
Ethionamide	2.50				1		1						
Ethionamide	5.00	23		23	3		3	2		2			
Ethionamide	10.00	4		4									
Kanamycin	0.00										1		1
Kanamycin	2.50				1		1						
Kanamycin	5.00	11		11	2		2						
Kanamycin	6.00	12		12									
Capreomycin	0.00										1		1
Capreomycin	1.25				1		1						
Capreomycin	2.50				1		1						
Capreomycin	3.00							2		2			
Capreomycin	5.00				4		4						
Capreomycin	10.00	18	2†	20									
Cycloserine	30.00	8	2	10									
Cycloserine	60.00	2		2									

* VersaTREK®, Hain GenoType®, or both

† Includes borderline results

Table 4 continued: *M. tuberculosis,* Isolate D– resistant to Isoniazid at 0.2µg/ml and 1.0µg/ml, Streptomycin at 2.0µg/ml and 10.0µg/ml

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Results*		
Drug	Conc.	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
p-Aminosalicylic acid	2.00	7	11	18									
p-Aminosalicylic acid	4.00					1	1						
p-Aminosalicylic acid	8.00	2		2									
p-Aminosalicylic acid	10.00	2	3	5									
Amikacin	0.00										1		1
Amikacin	1.50							2		2			
Amikacin	2.00	1		1									
Amikacin	2.50				1		1						
Amikacin	4.00	3		3									
Amikacin	5.00	1		1	1		1						
Amikacin	6.00	7		7									
Amikacin	12.00	2		2									
Ofloxacin	0.00										1		1
Ofloxacin	1.00	2		2	1		1						
Ofloxacin	1.25				1		1						
Ofloxacin	2.00	15		15	4		4	1		1			
Ofloxacin	4.00	1		1	1		1						
Clofazimine	0.50				1		1						
Clofazimine	1.00	1		1									
Rifabutin	0.50	7		7	2		2						
Rifabutin	1.00	2		2	1		1						
Rifabutin	2.00	8		8									
Ciprofloxacin	1.00	2		2	1		1						
Ciprofloxacin	2.00	5	1	6	2		2						
Ciprofloxacin	4.00				1		1						
Levofloxacin	1.00	1		1									
Levofloxacin	1.50							2		2			
Levofloxacin	2.00				2		2						
Moxifloxacin	0.25							1		1			
Moxifloxacin	1.00	3		3									

* VersaTREK®, Hain GenoType®, or both

Isolate E, *M. tuberculosis*–resistant to Rifampin at 1.0µg/ml by Agar Proportion

Rifampin is a first-line drug for treatment of all forms of tuberculosis caused by organisms known or presumed to be susceptible to this drug. It is bactericidal for *M. tuberculosis* at the critical concentration of 1.0µg/ml for AP (on Middlebrook 7H10 and 7H11 agars) and equivalent critical concentrations for BACTEC™, MGIT™, and VersaTREK® of 2.0µg/ml, 1.0 µg/ml, and 1.0µg/ml, respectively. The mechanism of action of RMP is to inhibit mycobacterial transcription by targeting DNA-dependent RNA polymerase. More than 96% of RMP-resistant isolates contain a mutation in the 81-base pair (bp) central region of the *rpoB* gene that encodes the β-subunit of the bacterial DNA-dependent RNA polymerase.[7] The activity of RMP in RMP-resistant isolates depends on both the mutation position and the type of amino acid change in the *rpoB* gene. Mutations in codons 531, 526, and 516 are among the most frequent mutations in RMP-resistant isolates and serve as predictors of RMP resistance. This isolate does not have a mutation in *rpoB*.

Of the 94 laboratories reporting RMP results for this isolate (some laboratories submitted results from more than one method) at the critical concentrations, this isolate was reported resistant by

- 100% (28/28) of the laboratories reporting AP results;
- 96% (22/23) of the laboratories reporting BACTEC™ results;
- 97% (61/63) of the laboratories reporting MGIT™ results; and
- 100% (4/4) of the laboratories reporting VersaTREK® results.

Two labs using molecular methods (one Hain and one XPERT Cepheid) reported this isolate as susceptible, which is consistent with the lack of an *rpo*B mutation by DNA sequence analysis.

Rifabutin
Seven laboratories tested RBT in at the critical concentration of 0.5µg/ml by AP. All reported resistance.

See Table 5 for the complete results submitted by all participants for Isolate E.

Table 5: Participant results for *M. tuberculosis*, Isolate E–resistant to Rifampin at 1.0µg/ml by AP

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Results*		
Drug	Conc.	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
Isoniazid	0.00										1		1
Isoniazid	0.10				22	1	23	61	1	62	4		4
Isoniazid	0.20	22		22				1		1			
Isoniazid	0.40				8		8	22		22	4		4
Isoniazid	1.00	23		23	1		1						
Isoniazid	4.00							1		1			
Isoniazid	5.00	3		3									
Rifampin	0.00										2		2
Rifampin	1.00		28	28		3	3	2	61	63		4	4
Rifampin	2.00				1	22	23		1	1			
Rifampin	5.00		4†	4		1	1						
Rifampin	10.00					1	1						
Pyrazinamide	20.00				1		1						
Pyrazinamide	100.00	1		1	16		16	58	1	59			
Pyrazinamide	300.00				1		1				2		2
Pyrazinamide	900.00				1		1						
Ethambutol	0.00										1		1
Ethambutol	2.50				18	3	21	1		1			
Ethambutol	5.00	21	2†	23	3		3	59	1	60	5		5
Ethambutol	7.50	2		2	3		3	1	1	2			
Ethambutol	8.00										4		4
Ethambutol	10.00	12		12									
Streptomycin	1.00				1		1	46	1	47	1		1
Streptomycin	2.00	27		27	18	1	19	1		1	1		1
Streptomycin	4.00	1		1				5	1	6			
Streptomycin	6.00				2		2						
Streptomycin	10.00	22		22									
Ethionamide	1.25				1		1						
Ethionamide	2.50				1		1						
Ethionamide	5.00	23		23	3		3	2		2			
Ethionamide	10.00	3		3									
Kanamycin	0.00										1		1
Kanamycin	2.50				1		1						
Kanamycin	3.00	1		1									
Kanamycin	5.00	10		10	1		1						
Kanamycin	6.00	11		11									
Capreomycin	0.00										1		1
Capreomycin	2.50				1		1						
Capreomycin	3.00							2		2			
Capreomycin	5.00				4		4						
Capreomycin	10.00	19		19									
Cycloserine	30.00	10		10									
Cycloserine	60.00	2		2									

* VersaTREK®, Hain GenoType®, or both

† Includes borderline results

Table 5 continued: *M. tuberculosis,* Isolate E–resistant to resistant to Rifampin at 1.0µg/ml

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Results*		
Drug	Conc.	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
p-Aminosalicylic acid	2.00	18		18									
p-Aminosalicylic acid	4.00				1		1						
p-Aminosalicylic acid	8.00	2		2									
p-Aminosalicylic acid	10.00	5		5									
Amikacin	0.00										1		1
Amikacin	1.50							2		2			
Amikacin	2.00	1		1									
Amikacin	2.50				1		1						
Amikacin	4.00	2		2									
Amikacin	5.00	1		1	1		1						
Amikacin	6.00	7		7									
Amikacin	12.00	2		2									
Ofloxacin	0.00										1		1
Ofloxacin	1.00	2		2	1		1						
Ofloxacin	1.25				1		1						
Ofloxacin	2.00	14		14	3		3						
Ofloxacin	4.00	1		1	1		1						
Clofazimine	1.00	1		1									
Rifabutin	0.05					1	1						
Rifabutin	0.25					1	1						
Rifabutin	0.50		7	7		3	3						
Rifabutin	1.00		2	2		1	1						
Rifabutin	2.00	3	4	7									
Ciprofloxacin	1.00	2		2	1		1						
Ciprofloxacin	1.25				1		1						
Ciprofloxacin	2.00	4	1	5	2		2						
Ciprofloxacin	2.50				1		1						
Ciprofloxacin	4.00				1		1						
Ciprofloxacin	5.00				1		1						
Levofloxacin	1.00	1		1									
Levofloxacin	1.50							2		2			
Levofloxacin	2.00				2		2						
Moxifloxacin	0.25							1		1			
Moxifloxacin	1.00	3		3									
Clarithromycin	3.00		1	1									
Azithromycin	3.00		1	1									

* VersaTREK®, Hain GenoType®, or both
† Includes borderline results

Abbreviations Used in This Report

AMK	amikacin
AP	agar proportion
BACTEC™	BACTEC™ 460TB
bp	base pair
BSL	Biosafety Level
CDC	Centers for Disease Control and Prevention (CDC)
CIP	ciprofloxacin
CLF	clofazimine
CLSI	Clinical Laboratory and Standards Institute
CM	capreomycin
CS	cycloserine
DNA	deoxyribonucleic acid
DST	Drug Susceptibility Testing
EMB	ethambutol
HMO	Health Maintenance Organization
INH	isoniazid
KM	kanamycin
LEV	levofloxacin
MGIT™	BACTEC™ MGIT™ 960 (Mycobacteria Growth Indicator Tube)
MOX	moxifloxacin
MPEP MTB NTM DST	Model Performance Evaluation Program for *Mycobacterium tuberculosis* and Nontuberculous Mycobacteria Drug Susceptibility Testing
NIH	National Institutes of Health
NTM	Nontuberculous Mycobacteria
OFX	ofloxacin
PAS	p-aminosalicyclic acid
PPO	Preferred Provider Organization
PZA	pyrazinamide
QRDR	quinolone-resistance-determining region
RBT	rifabutin
RMP	rifampin
RNA	ribonucleic acid
SM	streptomycin
ETH	ethionamide
VersaTREK®	VersaTREK®Myco Susceptibility Kit

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